Aberrant expression of long non-coding RNAs in peripheral blood mononuclear cells isolated from patients with gouty arthritis

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Abstract. Gouty arthritis (GA) is the most common inflammatory and immune-associated disease, and its prevalence and incidence exhibit yearly increases. The aim of the present study was to analyse the expression profile variation of long non-coding RNAs (lncRNAs) in GA patients and to explore the role of lncRNAs in the pathogenesis of GA. The peripheral blood mononuclear cells of GA patients and of healthy controls (HCs) were used to detect for the differentially expressed lncRNAs by microarray. The functional annotations and classifications of the differentially expressed transcripts were predicted using Gene Ontology (GO) and pathway analysis. The results were then verified by reverse transcription-quantitative (RT-q)PCR. A total of 1,815 lncRNAs and 971 mRNAs with a >2-fold difference in the levels of expression in the GA patients compared with those in the HCs were identified. According to the GO functional enrichment analysis, the differentially expressed lncRNAs were accumulated in terms including protein binding, catalytic activity and molecular transducer activity. The pathways predicted to be involved were the tumor necrosis factor signaling pathway, osteoclast differentiation, NOD-like receptor signaling pathway and NF-κB signaling pathway. The expression of six lncRNAs was measured by RT-qPCR and the results were consistent with those of the microarrays. Among these lncRNAs, AJ227913 was the most differentially expressed lncRNA in GA patients vs. HCs. The expression of several lncRNAs was significantly changed in GA patients compared with that in HCs, which suggests that these lncRNAs with differential expression levels may have an important role in the development and progression of GA.

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Introduction

Gouty arthritis (GA) is one of the most common types of inflammatory arthritis and is caused by the deposition of monosodium urate (MSU) crystals in and around joints. Elevated serum urate levels are recognized as an important risk factor for the development of GA (1). However, only ~10% of individuals with hyperuricaemia actually develop GA. Therefore, it has been suggested that hyperuricemia alone is not sufficient to cause GA (2). Although previous studies have identified several candidate risk genes (SLC2A9, ABCG2 and URAT1) associated with raised serum urate concentrations that increase the risk for an individual to develop GA (3-5), the exact pathogenesis of gout remains elusive. There may be other genes that are not associated with urate metabolism that also contribute to susceptibility to this disease.

Long non-coding RNAs (lncRNAs) are a group of RNA transcripts that are >200 nt in length. Initially, they were thought to be 'transcriptional noise' due to their lack of significant open reading frames and protein-coding ability. The human genome encoded tens of thousands of lncRNAs (6). In the past decade, an increasing number of lncRNAs have been characterized. Certain lncRNAs have been indicated to have important roles in diverse biological processes, including cell development, tumorigenesis and immune response. lncRNAs exert critical functions in the transcriptional, post-transcriptional and epigenetic regulation of gene expression (7). Although several studies have identified lncRNAs involved in a series of human biological and disease-associated processes (8,9), only isolated examples on the regulation of rheumatic diseases through lncRNAs have been provided and the roles of lncRNAs in the progression of GA have remained to be fully elucidated.

In the present study, lncRNA microarrays were performed to evaluate the global expression profile of lncRNAs in

peripheral blood mononuclear cells (PBMCs) of GA patients. In a subsequent Bioinformatics analysis, the lncRNA functions were annotated by Gene Ontology (GO) analysis, pathway analysis and network analysis. Reverse transcription-quantitative (RT-q)PCR was used to validate several random lncRNAs that were upregulated and downregulated in the GA patients compared with those in the healthy controls (HCs). These results provide information for future studies on GA.

Patients and methods

Study population and ethical statement. All participants were recruited from the Department of Rheumatology of the Affiliated Hospital of North Sichuan Medical College (Nanchong, China) between February 2015 and July 2016. The study group consisted of 60 GA patients and 60 HC subjects. The diagnoses of GA were confirmed by a clinical endocrinology physician, according to the American College of Rheumatology classification criteria (10). The patients had not received any systemic anti-inflammatory treatments or drugs to control the production and elimination of uric acid prior to obtainment of the blood samples. According to the design of a matched case-control study, healthy subjects with no history of gout and without any systemic inflammatory disease were enrolled in the present study. All the participants were of Chinese Han descent. Blood samples from all participants were obtained in the morning following overnight fasting for at least 12 h, and were collected in sterile, single-use, anticoagulant-coated tubes and immediately sent to the laboratory for genetic testing.

RNA isolation. Ficoll-Hypaque density gradient centrifugation was performed to isolate the PBMCs from total blood samples. Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA quantity and quality were measured using a NanoDrop ND-2000c spectrophotometer (Thermo Fisher Scientific, Inc.) All of the RNA samples were stored at -80˚C until further use.

Microarray analysis. Samples from three GA patients and three HC subjects were used for the lncRNA microarray analysis on an Agilent SurePrint G3 Gene Expression Microarrays for Human (v3) platform (part no. G4851C; Agilent Technologies). The RNA integrity of these samples was assessed with an Agilent Bioanalyzer 2100 (Agilent Technologies). In this study, RNA integrity number (RIN) and 28S to 18S rRNA ratio (28S/18S) were used as a criterion for RNA quality. Only samples with 2,100 RIN ≥7.0 and 28S/18S ≥0.7 were used. Total RNA was amplified and labelled with a Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies), following the manufacturer's protocol. Labelled cRNA (pmol Cy3/μg cRNA) was purified with an RNeasy mini kit (Qiagen GmBH). Each slide was hybridized with 1.65 μg Cy3-labelled cRNA using a Gene Expression Hybridization Kit (Agilent Technologies) in a hybridization oven (Agilent Technologies) according to the manufacturer's protocol. After 17 h of hybridization, slides were washed in staining dishes (Thermo Fisher Scientific, Inc.) with a Gene Expression Wash Buffer Kit (Agilent Technologies) following the manufacturer's protocol. Slides were scanned with an Agilent microarray scanner (Agilent Technologies) with default settings (dye channel: Green, scan resolution=3 μm, 20 bit). Data were extracted with Feature Extraction software 10.7 (Agilent Technologies). Raw data were normalized with a Quantile algorithm, with Gene Spring Software 11.0 (Agilent Technologies). Differentially expressed lncRNAs and mRNAs between the two groups were identified when fold-change ≥2.

'Cis' and 'Trans' analysis of lncRNAs. The cis role of lncRNA refers to the lncRNA acting on neighbouring target genes (11,12). The protein-coding genes 10 kbp upstream or downstream of the lncRNAs were screened as potential 'cis'-interacting genes. The trans predictions were made using blast to identify complementary or similar sequences. The complementary energy between the two sequences was calculated by using RNAplex. The genes with e ≤-30 were selected as potential 'trans'-interacting genes (13).

Gene Ontology (GO) enrichment and pathway analysis. GO analysis was used to identify functional terms in different categories enriched by the differentially expressed mRNAs (www.geneontology.org; release 2016-08-08) (14). The KEGG database (www.genome.jp/kegg; release 79, 2016/07) was used to determine the biological pathways that were enriched by the differentially expressed mRNAs (15).

RT-qPCR assay. The lncRNAs selected and the sequences of the primers that were used for qPCR are provided in Table I. GAPDH was used as the endogenous control. In brief, total RNA was extracted as described above and then reverse-transcribed into complementary (c)DNA using a PrimeScript® RT Reagent kit with gDNA Eraser (Perfect Real Time; Takara Bio Inc.) following the manufacturer's protocol. The differential expression of lncRNAs that were identified in the microarray analysis was measured by qPCR using SYBR Green assays (Takara Bio Inc.). The reactions were performed in a final volume of 20 μl and included 10 μl Power SYBR Green PCR Master Mix, 0.4 μl ROX Dye, 0.5 μl final working concentration of 10 pmol/l of each of the forward and reverse primers, 2 μl cDNA and 6.6 μl nuclease-free water. The reactions were performed using a QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for PCR were as follows: Pre-denaturation at 95˚C for 10 min, and amplification for 40 cycles of denaturation at 95˚C for 15 sec and elongation at 60˚C for 1 min. All the experiments were performed in duplicate and the mean value was used for further analysis. Relative lncRNA concentrations were calculated using the $2^{-\Delta\Delta Cq}$ method (16).

Statistical analysis. Statistical analysis was performed using the SPSS 22.0 software package (IBM Corp.). To analyse differences in expression of individual lncRNAs or mRNAs in the microarray analysis, Student's t-tests were used. Regarding the results of the PCR analysis, the significance of differences in expression levels of lncRNAs were determined by the Kruskal-Wallis test or Mann-Whitney U-test. For statistical correlation, Spearman correlation coefficient was used. Fisher's exact test was used in the GO enrichment and KEGG pathway analyses. $P<0.05$ was considered to indicate statistical significance.

Table I. Primer sequences used for validation of lncRNAs by reverse transcription-quantitative PCR.

lncRNA	Forward primer (5'-3')	Reverse primer (5'-3')	Length (bp)
AJ227913	TTTTGCCAAGGAGTGCTAAAGA	AACCCTCTGCACCCAGTTTTC	194
AK001903	CCAGCGGATATTTTGGTGTTTG	AGAAGCTATCAGGCGTTGCTG	86
ENSG00000239182	TAGCACTGTTGCCTGAGCCA	GGAAGGAGCAGCCCACAGC	95
lnc-AP000769.1-1	CAAGCAGAAGCAACAGGTCA	GAGCCAGGAAGATTGGAGAA	144
lnc-PCYOX1L-1:1	GGAAAGGCAGTAATCAACTCCA	ACTCCACAATCCCCACAGC	171
lnc-CCDC64B-1:8	ACCCCCACCCCAGGTCTTC	GCTGTGTCTCTGTCTTGGTCTCTT	268
GAPDH	ATCATCCCTGCCTCTACTG	AGTCAGAGGAGACCACCTG	241

lncRNA, long non-coding RNA.

Results

Overview of the lncRNA and mRNA profiles. The expression levels of the lncRNAs and mRNAs from three GA patients and paired control samples were analysed using lncRNA expression microarrays, which contained a total of 63,431 lncRNAs. All lncRNAs and mRNAs that were differentially expressed with a fold-change ≥2.0 between the GA and HC groups are provided in Tables SI and SII. Analysis of the microarray data revealed that 1,815 lncRNAs and 971 mRNAs were significantly differentially expressed in GA patients compared with those in the HC group. In total, there were 875 upregulated lncRNAs and 940 downregulated lncRNAs in GA patients compared with those in the HC group (Fig. 1A). A total of 465 mRNAs were upregulated and 506 mRNAs were downregulated in the GA patients compared with those in the HC group (Fig. 1B). Table II presents the 10 most upregulated and downregulated lncRNAs in the GA patients vs. HC group. Among these lncRNAs, AJ227913 (fold-change, 17.014) was the most upregulated lncRNA and 52094-1 (fold-change, 11.772) was the most downregulated lncRNA in the GA patients vs. HC group. Table III lists the 10 most upregulated and downregulated mRNAs. The most significantly upregulated mRNA was NM_005217 (fold-change, 18.208), and the most downregulated mRNA was NM_005353 (fold-change, 14.047) in the GA patients vs. HC group. Studies have suggested that several lncRNAs regulate their own transcription in 'cis', as well as that of nearby genes, by recruiting remodelling factors to local chromatin (11). Therefore, the chromosomal co-expression was then analysed. According to this analysis, the lncRNAs had 148 'cis' genes and 145 'trans' genes. The downregulated lncRNAs had 233 'cis' genes and 338 'trans' genes (detailed results are displayed in Tables SI and SII).

GO analysis. GO analysis was performed to identify the transcripts with terms that covered 3 domains (Fig. 2): Biological process, cellular component and molecular function. Fisher's exact test was employed to determine if there was more overlap between the differentially expressed list and the GO annotation list than what would be expected by chance (P<0.05 is recommended). The most highly enriched GO terms in the category molecular function were identified as 'binding', 'catalytic activity' and 'molecular transducer activity'. The most highly enriched GO terms in the category cellular component were 'cell' and 'cell part'. In the category biological process, the most highly enriched GO term was 'cellular process'. 'Metabolic process', and 'immune system process' were also significantly enriched.

Pathway analysis. KEGG pathway analysis was performed for the differentially expressed transcripts. The pathway analysis indicated which biological pathways were significantly enriched for the differentially expressed transcripts. A total of 19 pathways corresponded to the differentially expressed transcripts (Table IV). The gene category 'tumor necrosis factor (TNF) signaling pathway' was the most enriched network, promoting cell survival and differentiation, as well as immune and inflammatory responses (17). Furthermore, 8 transcripts were significantly enriched in the pathway 'osteoclast differentiation', which is closely linked to bone development and repair (18). Among these pathways, the 'NOD-like receptor signaling pathway' and 'NF-κB signaling pathway' (Fig. 3) have been previously demonstrated to be involved in MSU-mediated inflammation through activation of Toll-like receptors/MyD88-dependent NF-κB signaling, NLR family pyrin domain containing 3 inflammasome and interleukin (IL)-1β/MyD88-dependent IL-1 receptor signalling (19).

RT-qPCR validation. To validate the results of the microarray, four of the upregulated lncRNAs and two of the downregulated lncRNAs were selected for the RT-qPCR analysis of 60 GA patients and 60 HC subjects from a Han Chinese population. The results confirmed that the expression of AJ227913 (P<0.05), AK001903 (P<0.05), ENSG00000239182 (P<0.05) and lnc-AP000769.1-1 (P<0.05) was significantly increased in gouty arthritis patients compared with that in healthy subjects, whereas the expression of lnc-PCYOX1L-1:1 (P<0.05) and lnc-CCDC64B-1:8 (P<0.05) was significantly decreased in GA patients compared with that in healthy subjects. The fold changes of the normalized levels for the six lncRNAs were 17.02, 10.97, 7.71, 2.03, 2.31 and 2.05, respectively, in the gene chip analysis comparing the GA to the HC groups. Regarding the expression levels in the RT-qPCR analysis, these fold changes were 10.76, 7.64, 4.15, 3.16, 2.03 and 2.50, respectively, in the GA group compared to those in the control group. Thus, the results of the RT-qPCR and the microarray data were consistent (Fig. 4). Among the lncRNAs analysed, AJ227913 had the largest difference in the expression between the two

Table II. Top 10 significantly differentially expressed lncRNAs between gouty arthritis patients and healthy controls.

A, Upregulated lncRNAs

lncRNA name	Source database	Fold change	P-value
AJ227913	NONCODERv3	17.014	0.035
AK001903	NONCODERv3	10.971	0.016
THC2686626	Agilent_HUMAN_G3V2	9.332	0.034
TCONS_00026568	Agilent_HUMAN_G3V2	8.450	0.008
lnc-COL20A1	lncipedia2.1	7.833	0.038
ENST00000604411.1	gencode_v17	7.741	0.020
lnc-AP000769.1-1	lncipedia2.1	7.710	0.006
TCONS_00026396	Agilent_HUMAN_G3V2	7.607	0.008
TCONS_00018683	Agilent_HUMAN_G3V2	7.526	0.026
TCONS_l2_00003767	Agilent_HUMAN_G3V2	6.914	0.012

B, Downregulated lncRNAs

lncRNA name	Source database	Fold change	P-value
52094-1	UCSC	11.772	0.001
TCONS_l2_00000650	Agilent_HUMAN_G3V2	11.392	0.007
CR616125	NONCODERv3	9.367	0.012
lnc-KCNT2	lncipedia2.1	8.885	0.018
BX538226	NONCODERv3	8.794	0.001
ENST00000430519	Agilent_HUMAN_G3V2	8.410	0.025
lnc-APTX-1	lncipedia2.1	7.178	0.001
TCONS_l2_00007044	UCSC	7.000	0.033
37475-1	NONCODERv3	6.935	0.017
AK056081	Agilent_HUMAN_G3V2	6.894	0.048

lncRNA, long non-coding RNA; UCSC, University of California Santa Cruz.

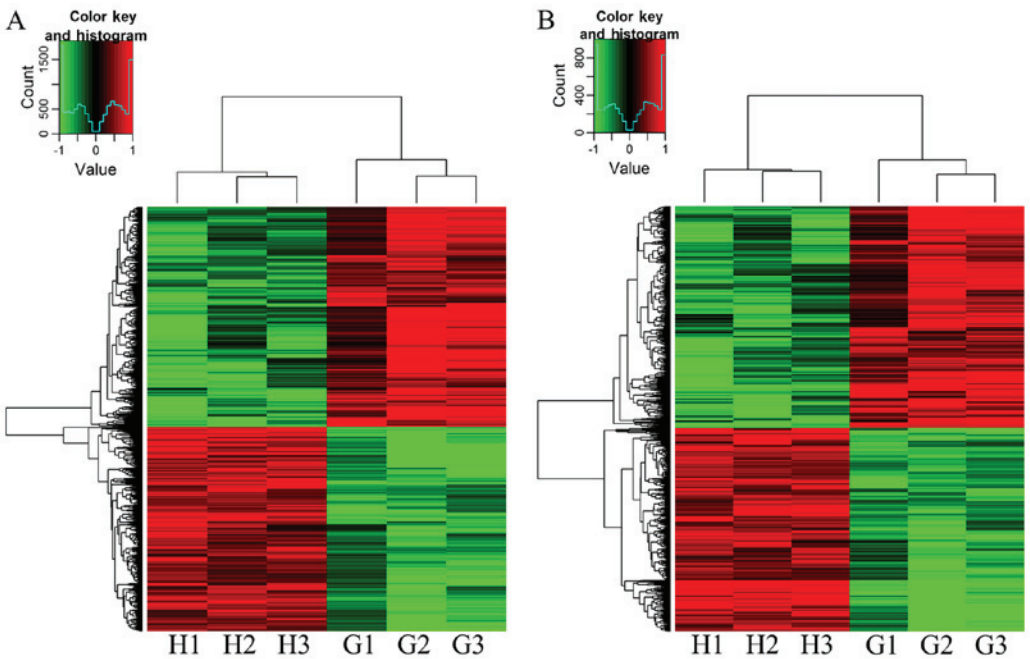

Figure 1. Expression profiling of (A) long non-coding RNAs and (B) mRNAs in GA patients and in healthy controls. Red and green colours represent up- and downregulated genes, respectively. G, GA patients; H, healthy controls. GA, gouty arthritis.

Table III. Top 10 significantly differentially expressed mRNAs between gouty arthritis patients and healthy controls.

A, Upregulated mRNAs

Primary accession number	Gene symbol	Fold change	P-value
NM_005217	DEFA3	18.208	0.050
NM_014364	GAPDHS	14.277	0.003
NM_173557	RNF152	11.779	0.032
NM_000584	IL8	11.616	0.030
DB514319	NA	11.600	0.028
NM_002192	INHBA	11.220	0.029
THC2725968	XLOC_002791	10.485	0.017
ENST00000511330	XLOC_003547	9.264	0.001
NM_003955	SOCS3	9.186	0.002
NM_001964	EGR1	8.8103	0.043

B, Downregulated mRNAs

Primary accession number	Gene symbol	Fold change	P-value
NM_005353	ITGAD	14.047	0.007
ENST00000435913	NA	8.365	0.016
NM_015267	CUX2	8.346	0.042
NR_026970	LY86-AS1	8.068	0.033
ENST00000400449	NA	7.909	0.014
AK095683	PSMG4	7.880	0.037
NM_021161	KCNK10	7.879	0.022
LNCA_33_P3384628	NA	7.670	0.010
NM_080747	KRT72	7.656	0.008
NR_040093	LOC100505678	7.343	0.016

DEFA3, defensin, α 3; GAPDHS, glyceraldehyde-3-phosphate dehydrogenase, testis-specific; RNF152, ring finger protein 152; IL-8, interleukin 8; INHBA, inhibin β A; SOCS, suppressor of cytokine signalling; EGR1, early growth response protein 1; CUX2, cut like homeobox 2; ITGAD, integrin α-D; LY86-AS1, LY86 antisense RNA 1; PSMG4, proteasome assembly chaperone 4; KCNK10, potassium channel subfamily K member 10; KRT72, keratin, type II cytoskeletal 72; NA, not available.

groups. Furthermore, Spearman correlation analysis suggested that the expression levels of AJ227913 in GA patients were correlated with urea (r=0.618, P<0.05), creatinine (r=0.382, P<0.05) and cystatin C (r=0.482, P<0.05; Table SIII).

Discussion

Protein-coding genes account for only ~2% of the human genome, while a large proportion of the genome is transcribed to generate non-coding RNAs that have been estimated to comprise up to 98-99% of the genome (20). It has become apparent that mutations and abnormal regulation of certain lncRNAs have important roles in the occurrence and development of various rheumatic diseases (21). Luo *et al* (22) indicated that 8,868 lncRNAs were highly differentially expressed in systemic lupus erythematosus (SLE) samples compared with those in a healthy group, and overall, aberrant expression profiles of lncRNAs may provide important insight into the pathogenesis of SLE (23). Downregulation of lnc-dendritic cell lncRNA (lnc-DC) in the plasma of patients with SLE may regulate type 17 T-helper cell differentiation by regulating the expression of signal transducer and activator of transcription 3; thus, the plasma levels of lnc-DC may be a potential biomarker for SLE (24,25). In rheumatoid arthritis (RA) patients, MALAT1 increases the expression of caspase-3 and caspase-9 and promotes cell growth and apoptosis of RA fibroblast-like synoviocyte cells. Methotrexate treatment increases the expression of lincRNA-p21 and inhibits NF-κB activity compared with those in untreated RA patients (26,27). Patients with osteoarthritis (OA) were indicated to have high levels of lncRNA-CIR. Treatment of chondrocytes *in vitro* with IL-1β and TNF-α significantly increased the expression of lncRNA-CIR compared with that in the controls. Furthermore, silencing of lncRNA-CIR by small interfering RNA reduced the expression of matrix metalloproteinase (MMP)13 and ADAM metallopeptidase with thrombospondin type 1 motif 5 compared with that in the controls. Thus, lncRNA-CIR may act as a potential target in OA therapy (28,29). To examine the functional implications of lncRNAs in GA, the expression profiles of lncRNAs

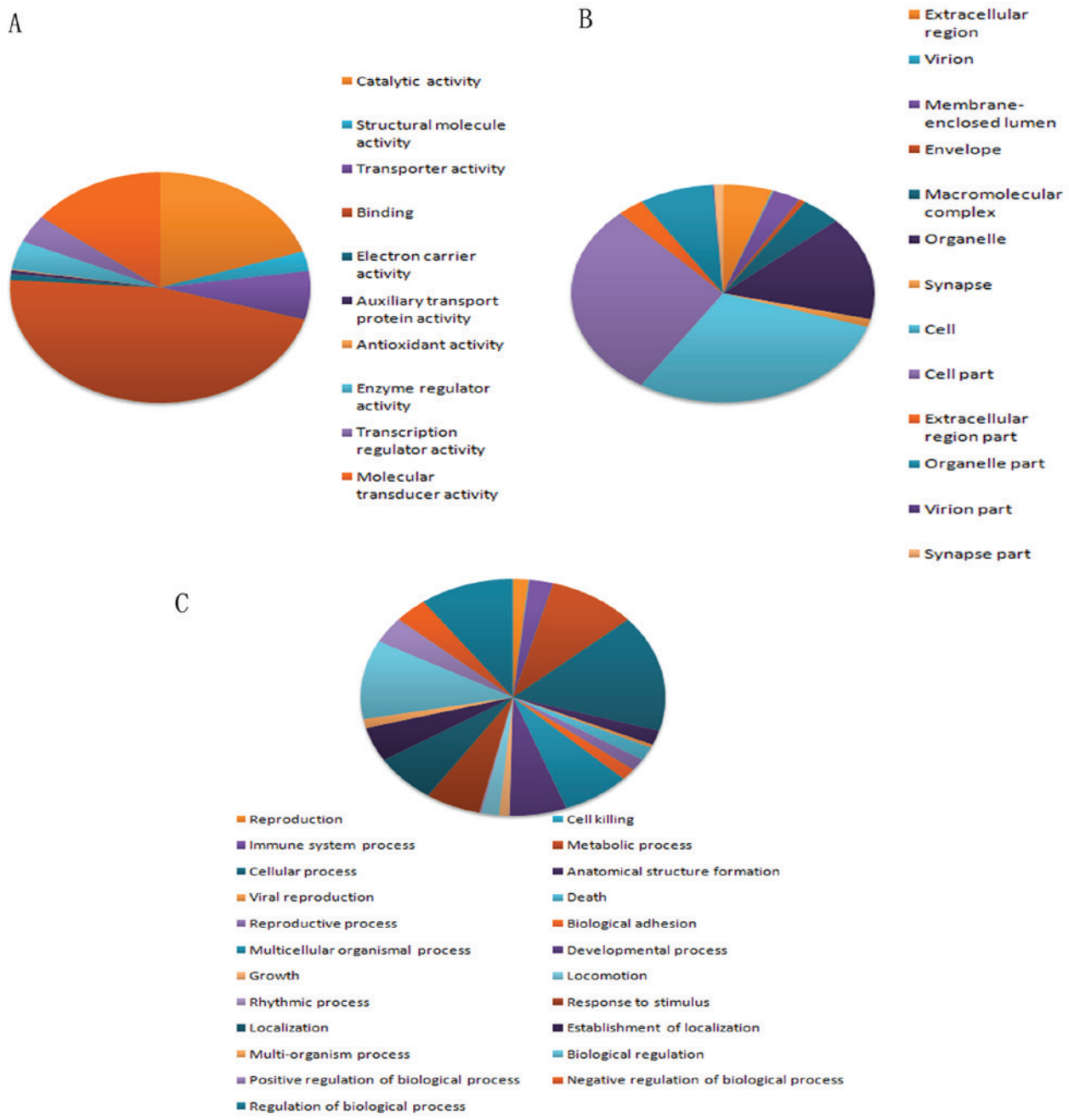

Figure 2. Gene Ontology analysis of the differentially expressed transcripts. (A) Molecular function. (B) Cellular component. (C) Biological process.

in GA patients and in HCs were determined using microarrays.

In the present study, the lncRNA expression profiles in 3 GA patients and 3 HCs comprising 63,431 lncRNAs were assessed by microarray analysis. A total of 875 lncRNAs were significantly upregulated and 940 lncRNAs were significantly downregulated, which was by >2-fold. A total of 6 lncRNAs, which were not previously reported by studies on other diseases, were then randomly selected for validation in 60 other GA patients and in paired HCs by RT-qPCR. The results of the RT-qPCR analysis were consistent with those of the microarray analysis. The differential expression of lncRNAs in GA implied that lncRNAs may have a crucial role in the onset and development of GA. However, further validations and functional studies are warranted in the future. In the present study, a significant increase in the expression level of AJ227913 in GA patients compared with that in HCs was identified based on the microarray analysis. The AJ227913 expression levels in GA patients had a close correlation with urea ($r=0.618$, $P<0.05$), creatinine ($r=0.382$, $P<0.05$) and cystatin C ($r=0.482$, $P<0.05$) levels based on the Spearman correlation analysis. Most circulating uric acid is freely filtered in the kidney. Urea, creatinine, cystatin C and uric acid are crude indicators of renal filtration and excretory function. However, uric acid is excreted in urine in healthy humans. Uric acid excretion may be impaired by kidney disease, leading to chronic hyperuricaemia. High uric acid levels in the body may lead to glomerular filtration disorders and a decrease in renal tubular secretion. It may increase the risk of gout or gouty nephropathy (30,31). These results suggest that AJ227913 may

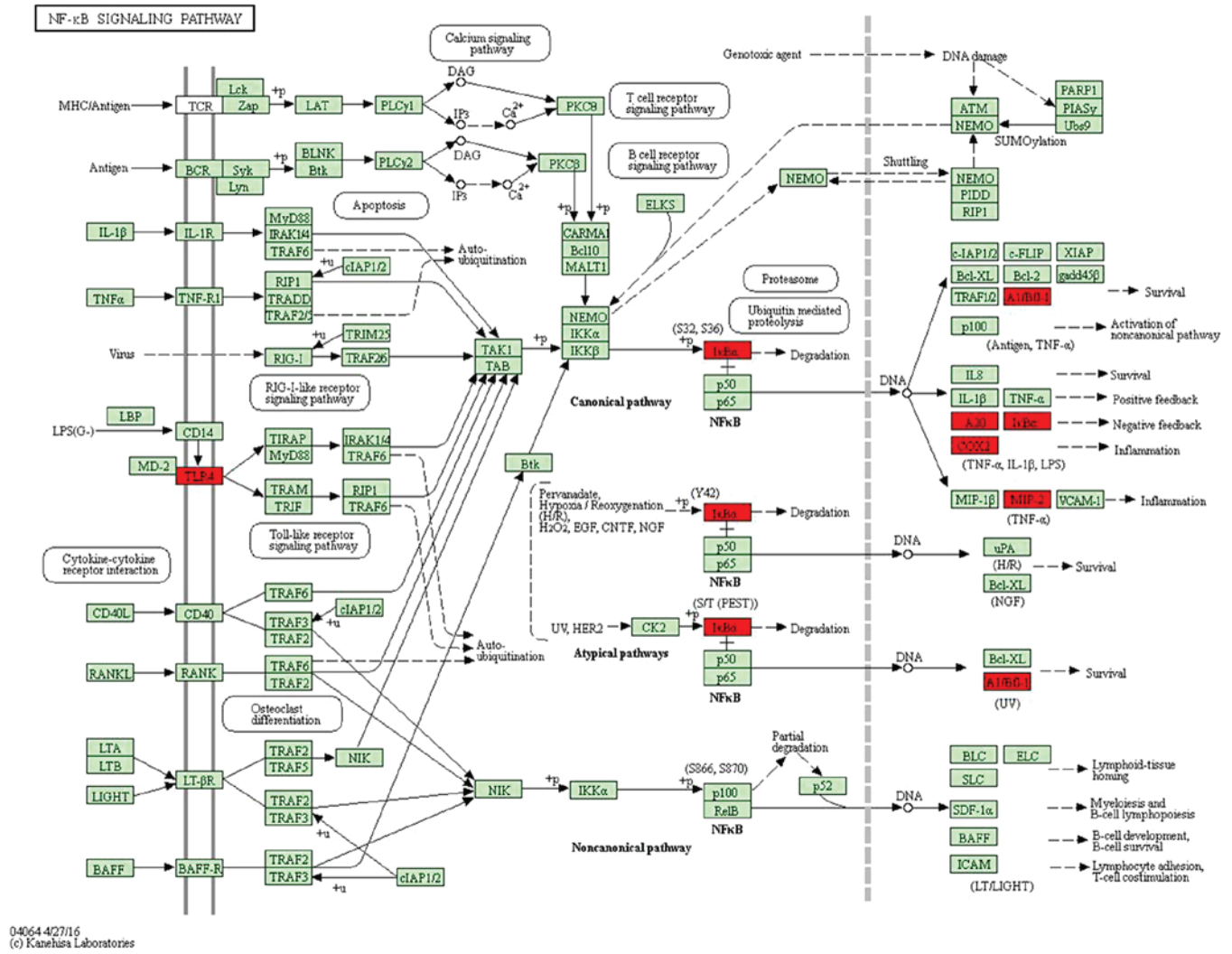

Figure 3. Schematic diagram of the gene category of the NF-κB signaling pathway.

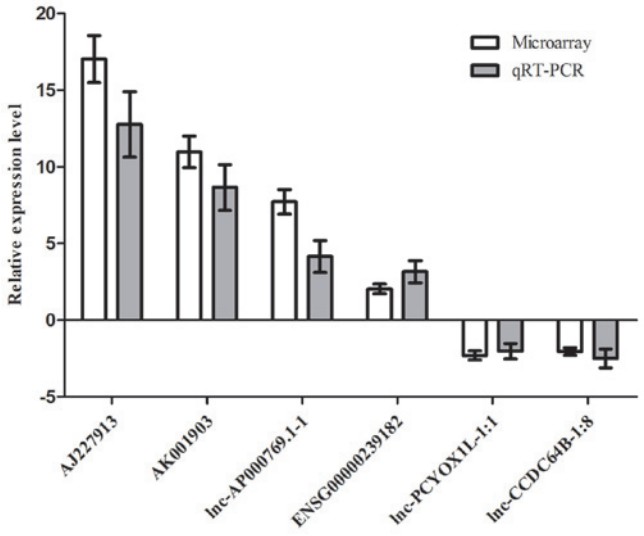

Figure 4. Comparison of the lncRNA expression levels between the microarray and RT-qPCR results. Six differentially expressed lncRNAs were validated by RT-qPCR. The Y-axis represents the log-transformed median fold change (gouty arthritis/healthy controls) in the expression across 60 samples. Data presented as the mean ± SD. The RT-qPCR results were consistent with those of the microarray data. lncRNA, long non-coding RNA; RT-qPCR, reverse transcription-quantitative PCR.

be involved in the dynamic balance of the production and elimination of uric acid in GA patients.

GO and pathway analyses were performed to predict the biological functions of the differentially expressed lncRNAs and potential mechanisms in GA progression. According to this analysis, the mostly highly enriched GO terms by the differentially expressed transcripts were binding, catalytic activity, molecular transducer activity, cellular process, cell and cell part. The GO project is a framework for modelling biology that may be used to describe the functional classification of differentially expressed transcripts. The pathway analysis suggested that the differentially expressed transcripts were associated with 19 pathways. The gene category 'TNF signaling pathway' promotes cell survival and differentiation, as well as immune and inflammatory responses (17). The 'osteoclast differentiation' process is known to be important for bone development and repair (18). In addition, the 'NOD-like receptor signaling pathway' and 'NF-κB signaling pathway' have been reported to participate in MSU-mediated signaling cascades, which induce inflammasome-dependent gouty inflammation (19). These results have prompted us to investigate the molecular mechanisms of the pathogenesis of gout, which is important for further studies.

Table IV. KEGG pathway analysis of differentially expressed transcripts.

Term	Description	Count	P-value	Enrichment score
hsa05134	Legionellosis	6	0.001	5.217
hsa04621	NOD-like receptor signaling pathway	6	0.001	5.034
hsa04610	Complement and coagulation cascades	7	0.001	4.238
hsa05150	Staphylococcus aureus infection	5	0.007	4.195
hsa04668	TNF signaling pathway	11	0.000	3.913
hsa05140	Leishmaniasis	6	0.004	3.878
hsa04978	Mineral absorption	4	0.023	3.679
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	5	0.014	3.516
hsa04012	ErbB signaling pathway	6	0.010	3.261
hsa05133	Pertussis	5	0.020	3.188
hsa04064	NF-κB signaling pathway	10	0.013	3.085
hsa05131	Shigellosis	4	0.047	2.943
hsa04380	Osteoclast differentiation	8	0.006	2.898
hsa05132	Salmonella infection	5	0.034	2.780
hsa05142	Chagas disease (American trypanosomiasis)	6	0.021	2.759
hsa05161	Hepatitis B	7	0.033	2.293
hsa04024	cAMP signaling pathway	9	0.023	2.163
hsa05205	Proteoglycans in cancer	9	0.028	2.100
hsa05166	HTLV-I infection	8	0.020	2.031

Hsa, *Homo sapiens*; ErbB, epidermal growth factor; NOD, nucleotide binding oligomerization domain; TNF, tumor necrosis fator; NF-κB, nuclear factor κB; cAMP, cyclic adenosine monophosphate; HTLV-I, human T-lymphotropic virus 1.

Gene expression is a highly complex, regulated process with numerous levels of regulation (epigenetic, transcriptional, post-transcriptional, translational and post-translational). lncRNAs act as essential regulators of gene expression at the epigenetic level. The results of the 'cis' analysis performed in the present study suggested that AJ227913 may regulate IL-8 expression. IL-8 is a member of the chemokine family and is a key inflammatory mediator, which has an important role in regulating inflammation and immunity in various rheumatic diseases. Significantly higher concentrations of IL-8 were detected in the synovial fluid of RA patients than in those of HCs. IL-8 may stimulate neutrophils to produce cartilage-degrading enzymes, which may lead to joint tissue damage in RA. Inhibiting IL-8 production has been reported to reduce joint damage, which may be a novel target for the treatment of RA (32-34). Previous studies have indicated a marked increase in IL-8 in the exudate from patients with psoriatic arthritis (PsA) compared with that in the controls. An IL-8 monoclonal antibody was approved as a topical treatment for PsA in China. The therapeutic effect of anti-IL-8 is thought to be associated with a decrease in the accumulation of neutrophils and inflammatory cells, leading to the reduction of inflammation in patients with PsA (35-37). These results suggest a pathogenic role for IL-8 in rheumatic diseases. GA is a type of inflammatory arthritis that is caused by the deposition of MSU crystals. Several lines of evidence indicate that IL-8 is an essential mediator of neutrophil-mediated acute inflammation. The levels of IL-8 are increased during the acute and intercritical phases of GA. MSU may stimulate the secretion of IL-8 by human neutrophils. In rabbits with MSU crystal-induced arthritis, intra-articular injection of anti-IL-8 significantly attenuated crystal-induced joint swelling (38-40). Therefore, it was hypothesized that AJ227913 may regulate IL-8 expression in GA patients, and is thus involved in the pathogenesis of GA. Further research focusing on the roles of AJ227913 may provide a novel strategy for the treatment of GA.

In summary, the present study determined the expression profiles of patients with GA vs. HCs using microarray and identified a collection of differentially expressed lncRNAs. To the best of our knowledge, the present study was the first to examine the lncRNA profiles in GA. The present results may indicate that the differential expression of various lncRNAs has an important role in the development and progression of GA. Further studies will focus on the biological function of lncRNAs involved in GA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XZ conducted the majority of the experiments and wrote the manuscript. XG and JZ conceived and designed the study. YP and HL performed microarray analysis. CY and JL performed PCR assays. QY, YH and YQ collected blood samples and analyzed the clinical data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Affiliated Hospital of the North Sichuan Medical College (Nantong, China; IRB: 2015-EA-016). All of the participants who participated in the study provided written informed consent at the time of enrolment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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